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THE SYSTEMATICS OF *LOBOPHORA* (DICTYOTALES, PHAEOPHYCEAE) IN THE WESTERN ATLANTIC AND EASTERN PACIFIC OCEANS: EIGHT NEW SPECIES[1]

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Lobophora **is a common tropical to temperate genus of brown algae found in a plethora of habitats including shallow and deep-water coral reefs, rocky shores, mangroves, seagrass beds, and rhodoliths beds. Recent molecular studies have revealed that *Lobophora* species diversity has been severely underestimated. Current estimates of the species numbers range from 100 to 140 species with a suggested center of diversity in the Central Indo-Pacific. This study used three molecular markers (*cox*3, *rbc*L, *psb*A), different single-marker species delimitation methods (GMYC, ABGD, PTP), and morphological evidence to evaluate *Lobophora* species diversity in the Western Atlantic and the Eastern Pacific oceans. *Cox*3 provided the greatest number of primary species hypotheses(PSH), followed by *rbc*L and then *psb*A. GMYC species delimitation analysis was the most conservative across all three markers, followed by PTP, and then ABGD. The most informative diagnostic morphological characters were thallus thickness and number of cell layers in both the medulla and the dorsal/ventral cortices. Following a consensus approach, 14 distinct *Lobophora* species were identified in the Western Atlantic and five in the Eastern Pacific. Eight new species from these two oceans were herein described: *L. adpressa* sp. nov., *L. cocoensis* sp. nov., *L. colombiana* sp. nov., *L. crispata* sp. nov., *L. delicata* sp. nov., *L. dispersa* sp. nov., *L. panamensis* sp. nov., and *L. tortugensis* sp. nov. This study showed that the best approach to confidently identify *Lobophora* species is to analyze DNA sequences (preferably *cox*3 and *rbc*L) followed by comparative morphological and geographical assessment.**

Key index words: **biodiversity; *cox*3; cryptic diversity; *psb*A; *rbc*L**

Abbreviations**: BS, bootstrap value; EP, Eastern Pacific Ocean; NW, Northwest; PP, posterior probability; PSH, Primary Species Hypotheses; SE, Southeast; WA, Western Atlantic Ocean**

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Lobophora (Dictyotaceae, Dictyotales) is one of the most common genera of marine brown algae. The genus can be found growing in a variety of habitats, from tropical to temperate reefs and rocky shores (Taylor 1960, van den Hoek et al. 1978, De Ruyter van Steveninck et al. 1988, Vieira et al. 2017), and from the intertidal region to depths of 82 m recorded in Puerto Rico (Ballantine et al. 2016), 61–107 m in the Bahamas (Ballantine and Aponte 2003, Slattery and Lesser 2014), and 90 m in the Gulf of Mexico (this study). *Lobophora* thalli are predominately decumbent or crustose, but they also occur as erect, fan-shaped, ruffled or dichotomously branched thalli. For example, an undescribed species has been reported growing epiphytically on the prop roots of red mangroves in Belize (Coen and Tanner 1989), and *L. variegata* has also been found growing on *Thalassia testudinum* leaves in the Florida Keys (Vieira et al. 2016).

The genus is ecologically important in tropical reef systems where it is an efficient competitor with corals for space (e.g., Slattery and Lesser 2014, Vieira et al. 2015). For example, *Lobophora* populations drastically increased following disturbances that impacted herbivores and corals in the Caribbean during the mid-1980s, making the genus a potential bio-indicator of coral reef health (e.g., De Ruyter van Steveninck and Breeman 1987, Hughes 1994, Diaz-Pulido et al. 2009, Lesser and Slattery 2011, Slattery and Lesser 2014). Despite its ecological importance in many marine communities (Jompa and McCook 2002, Diaz-Pulido et al. 2009), *Lobophora* has received much less taxonomic attention than other brown algae. This neglect is perhaps a result of *Lobophora*'s perceived simple and relatively similar thallus morphology observed across all previously known species. The generitype species is *Lobophora nigrescens* (Agardh 1894) described from Dromana Bay, Victoria, Australia. However, before molecular techniques were applied, virtually all specimens reported around the world, including specimens currently regarded as *L. nigrescens*, were referred to as "*L. variegata* (J.V. Lamouroux) Womersley ex Oliveira, 1977," a species now known to be restricted to parts of the Caribbean (Vieira et al. 2016).

Prior to Sun et al. (2012), the first molecular taxonomic study on *Lobophora*, only six species were described based on morphological traits alone. Recent molecular studies strongly suggested that *Lobophora* species diversity was highly underestimated. The current known global *Lobophora* diversity is appraised to be in the range of 100–140 species (Vieira et al. 2016) but to date only 28 have been formally described (Guiry and Guiry 2018). The remaining molecularly defined species continue undescribed, contributing to what is known as "dark taxa" (Page 2016). The highest species diversity of *Lobophora* is the Central Indo-Pacific, while only 14 species are reported for the Western Atlantic Ocean (WA) and five in the Eastern Pacific Ocean (EP; Schultz et al. 2015, Vieira et al. 2016, 2017).

This study focused on improving our understanding of *Lobophora* species diversity and taxonomy by thoroughly describing a range of molecularly defined species from the WA and the EP. We used a range of different molecular markers, different single-marker delimitation methods, and vegetative morphological evidence.

MATERIALS AND METHODS

Study area and collections. Specimens of *Lobophora* were collected in various tropical and subtropical coastal and offshore localities in the WA (i.e., North Carolina, Costa Rica, Gulf of Mexico, Panama, and Colombia) and EP (i.e., Mexico, El Salvador, Nicaragua, Easter Is., Costa Rica including Isla del Coco, and Panama). Some of the specimens used to describe new *Lobophora* species in this study (= LAF samples) were previously included in other *Lobophora* investigations (see Vieira et al. 2016, 2017; Table S1 in the Supporting Information). Collections were made from diverse habitats and from the intertidal to 90 m depths across different seasons, using snorkeling, SCUBA diving or by vessel-deployed Hourglass-design box dredging (Joyce and Williams 1969, Felder et al. 2014, Fredericq et al. 2014). *Lobophora* specimens, or portions thereof, were desiccated in silica gel for DNA extraction, dried as herbarium specimens for taxonomic vouchers, and liquid-preserved in 4% formalin-seawater for comparative anatomical and morphological analyses. Herbarium collections at MNHM (Santiago, Chile), USJ (San Jose, Costa Rica), and LAF (Lafayette, Louisiana) were by permission used to obtain DNA sequences and/or morphological assessments. Voucher specimens are deposited in LAF, WNC, and USJ (herbarium abbreviations follow Thiers 2017).

Molecular data acquisition. Silica gel-dried material was ground in liquid nitrogen and total genomic DNA was extracted using the DNeasy Plant mini Kit following the manufacturer's instructions (Qiagen, Hilden, Germany). DNA extraction from herbarium specimens was attempted using DNeasy Blood & Tissue Kit (Qiagen) and PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA, USA) as they were more efficient in older, or non-silica preserved, specimens. We chose to amplify two chloroplast genes (*rbc*L, *psb*A) and one mitochondrial gene (*cox*3) because of their successful use in recent *Lobophora* phylogenetic studies (e.g., Sun et al. 2012, Vieira et al. 2014, 2016). Information on primers, PCR, and sequencing conditions are listed in Table S2 in the Supporting Information. All DNA sequencing was done in-house at the University of Louisiana at Lafayette on an ABI Model 3130*xl* Genetic Analyzer (Life Technologies, Grand Island, NY, USA). Chromatograms were assembled using Sequencher 5.1 (Gene Codes Corp., Ann Arbor, MI, USA). All *cox*3, *rbc*L, and *psb*A *Lobophora* DNA sequences currently available in GenBank (Benson et al. 2009) were first downloaded and then sorted for distinct existing haplotypes. All distinct haplotypes were selected and included in this study. Sequence alignments for each marker were generated separately using Mega v.6.06 (Tamura et al. 2013).

Phylogenetic analyses. Maximum likelihood (ML) and Bayesian (BI) phylogenetic analyses were performed separately for each marker based on newly generated haplotypes combined with distinct publicly available haplotypes as described above. A codon-based partition analysis was conducted independently for each marker using the software PartitionFinder v.1.1.1 (Lanfear et al. 2012). Best partition schemes obtained

under the Akaike Information Criterion were the GTR + I + G for each independent codon position for both *cox*3 and *psb*A, and the GTR + G also for each codon position for the *rbc*L. These partitions were applied in all ML analyses as implemented in RAxML on the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). RAxML analyses used 1,000 restarts and 1,000 bootstrap (BS) replications. Results were visualized in FigTree v. 1.3.1 (Drummond and Rambaut 2009). Preliminary analyses indicated the need to use multiple outgroup taxa (data not shown) to stabilize tree topologies, hence several outgroups were used including species of *Padina*, *Dictyota*, *Dictyopteris*, *Zonaria*, and *Newhousia* in the Dictyotales, and *Microzonia* (=*Syringoderma*, see Camacho et al. 2018) in the Syringodermatales.

Bayesian analyses were constructed in BEAST v.1.8.3 (Drummond et al. 2012) using the partition schemes and respective model selections described above, an uncorrelated lognormal relaxed molecular clock, and a coalescent tree prior with other priors set to default. Markov Chain Monte Carlo (MCMC) analyses were run for 60 million generations for *cox*3 and *rbc*L, and 40 million for *psb*A, sampling trees every 1,000 generations. All results were inspected for convergence (ESS > 200) in Tracer v.1.6 (Rambaut et al. 2014), and based on this a burn-in of 10% was applied using TreeAnnotator v.1.8.3. Posterior probabilities (PP) were calculated based on a majority-rule consensus tree using all trees saved after the burn-in. Bayesian analyses were run both with outgroups to compare with ML trees, and without outgroups to generate ultrametric (strict) consensus trees needed in the General Mixed Yule Coalescent models (see below).

Species delimitation methods. Species delimitation analyses were conducted independently on the same alignments used for phylogenetic analyses, using three approaches: General Mixed Yule Coalescent (GMYC; Pons et al. 2006); Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012); and the Poisson Tree Processes (PTP; Zhang et al. 2013). These species delimitation methods (SDM) have been used previously to successfully delineate not only *Lobophora* species (e.g., Vieira et al. 2014, 2016, Schultz et al. 2015) but also species in other Dictyotales genera such as *Dictyota* (Tronholm et al. 2010), and *Padina* (Silberfeld et al. 2013).

GMYC analyses were executed through the package "Splits" in R (R Development Core Team 2014) using the multiple-thresholds option. PTP analyses used the ML trees with outgroups (Zhang et al. 2013) and were performed with the online implementation of this software (http://species.h-its.org) under the following parameters: 100,000 MCMC generations, thinning = 100, burn-in = 0.1 and seed = 123. For the ABGD analyses branch lengths were extracted from the ML trees using the function "cophenetic.phylo" of the package APE in R (Paradis et al. 2004, R Development Core Team 2014). These values were used to produce a distance matrix input file that was run with minimum (pmin) and maximum (pmax) intraspecific distance priors between 0.012% and 2% in 100 steps, and with a 0.05 relative gap width. Additionally, a plot of pairwise divergence, in terms of base pairs difference by gene length, was made in R using the package "Ape" (R Development Core Team 2014).

Concatenated phylogenetic analysis. A concatenated phylogeny of all three markers (*cox*3 + *rbc*L + *psb*A) was produced based on all species from the WA and EP, plus selected sister taxa as depicted in the three single marker phylogenies and single marker SDM. The concatenated tree compared an "all marker result" with the three independent single marker phylogenies. For the concatenated tree only a ML analysis was performed. Methods for model selection, ML parameters and software were the same as those implemented for single markers analyses described above. The evolutionary model selected was the GTR+G. Bootstrap values were based on 1,000 replications.

Morphological analyses. Comparative morphological character assessments of recently collected and dried herbarium specimens were carried out and correlated with the molecular-based results to establish new species delimitations and descriptions. Fragments of herbarium specimens (or in some cases silica gel-preserved material) were rehydrated by placing them in a 70/30 glycerin/water solution for 24 h. External characters included the habit (i.e., whether crustose, decumbent, erect, stipitate), size (length, width), and color. To access the internal anatomy, longitudinal and transverse sections of the middle portions of the thallus were made by hand using single and double-edged razor blades. The internal characters measured were: thallus thickness; total number of cell layers (medullary, ventral and dorsal cortical cell layers); height and length of ventral and dorsal subcortical (inner cortical) cells; and height, length, and width of medullary cells. Cortical cell layer counts included both outer and inner cortical cells. A total of 63 WA and EP specimens, ranging from two to 18 specimens per species (and 1–6 cross sections per specimen) were assessed for morphological and anatomical analyses. Digital photomicrographs were taken with a Canon EOS Rebel T2i camera (Melville, NY, USA) mounted on an Olympus BX60 microscope (Waltham, MA, USA). Measurements were made using the ImageJ program (https://imagej.nih.gov).

Taxonomic conclusions. Final taxonomic conclusions were based on the consensus of all molecular-based identification methods herein implemented, totaling 15 votes sensu Zhang et al. (2017): phylogenetic analyses (i.e., phylogenetically well-supported clades, 3 markers × 2 methods = 6 votes), and the single-marker SDM (3 markers × 3 methods = 9 votes). Molecular-based primary species hypotheses (PSH) were then corroborated by morphological and biogeographic data.

RESULTS

Lobophora *molecular datasets.* The *cox*3 alignment was 580 bp long, with 339 variable sites of which 273 were parsimony informative. The *rbc*L alignment was 1,157 bp long, with 446 variable sites of which 371 were parsimony informative. The *psb*A alignment was 866 bp long, with 287 variable sites of which 246 were parsimony informative. The concatenated alignment was 2871 bp long, with 954 variable sites of which 767 were parsimony informative. Most species had at least one specimen where all three markers were sequenced, except eight species for *cox*3, 15 for *rbc*L, and 26 for *psb*A.

Species delimitation methods. Global analysis: When all known *Lobophora* species and haplotypes are considered, different SDM applied to the three distinct markers yielded different numbers of primary species hypotheses (PSHs; Table 1). The base pairs substitution rate was found to be much higher in *cox*3 (mean: 0.130) than *rbc*L (mean: 0.076), and *psb*A (mean: 0.060; Fig. S1 in the Supporting Information).

*Cox*3 provided the greatest number of PSH ranging between 99 and 130, followed by *rbc*L (81–112 PSH), and *psb*A (54–71; Table 1). Species delimitation based on the GMYC turned out to be the most

TABLE 1. Total number of species resulting from the three different species delimitation analyses (GMYC, PTP, ABGD) based on the *cox*3, *rbc*L, and *psb*A across all *Lobophora* species datasets.

	*cox*3	*rbc*L	*psb*A
GMYC	(83-) 111 (-124)	(72-) 81 (-95)	(44-) 54 (-78)
PTP	99	87	71
ABGD	130	112	69

conservative across all three markers, followed by PTP, then by ABGD (Table 1). Detailed information from SDM of species not described in this study is available upon request.

The largest number of available sequences was for *cox*3 (n = 743), followed by *rbc*L (n = 472) and then by *psb*A (n = 383). Table S3 in the Supporting Information shows the percentage of identical haplotypes removed from each marker, and the total number of sequences used in the analyses.

Species delimitation methods. Western Atlantic and Eastern Pacific species: Previously published species delimitation analyses recognized 14 *Lobophora* species for the WA (*L. declerckii*; *L. canariensis*; *L. guadeloupensis*; *L. littlerorum*; *L. variegata*; *L. schneideri* = *L.* sp39; *L.* sp40; *L.* sp44; *L.* sp64; *L.* sp65; *L.* sp77; *L.* sp78; *L.* sp86; and *L.*spWA02) and five species for the EP (*L. sonderi*; *L. undulata*; *L.* sp21; *L.* sp22, and *L.* sp58) totaling 19 species for both regions (Schultz et al. 2015, Vieira et al. 2016, 2018). Quantitatively, our results recognized these 19 species, plus two completely new *Lobophora* lineages from the EP (LAF 06736 and LAF 06737) and one from the WA (*L.* sp44b = LAF 04331).

$GMYC_{cox3}$ found a total of 22 PSH for the WA and EP. *Lobophora* sp78 was the only WA and EP species without *cox*3 data. The total number of PSH with $ABGD_{cox3}$ was 27 by splitting *L.* sp21, *L.* sp44 complex, *L. declerckii*, *L. sonderi,* and *L. undulata* into different PSH (Fig. 1). PTP_{cox3} recovered 19 PSH and differed from $GMYC_{cox3}$ and $ABGD_{cox3}$ by grouping *L.* sp21 complex (*L.* sp21 + *L.* sp22 + *L.* sp. LAF 06736) into a single PSH, as it did for *L.* sp44 complex, *L. sonderi*, and *L. canariensis* with *L.* sp4 (Fig. 1).

Species delimitation based on the *rbc*L dataset was more conservative than the *cox*3 results identifying 16 (GMYC), 20 (ABGD), or 15 (PTP) PSHs. Four species clades lack *rbc*L data: *L. guadeloupensis*, *L. littlerorum*, *L.* sp64, and *L.* sp86. $GMYC_{rbcL}$ analysis clustered the *L.* sp21 complex into a single PSH as it did for the *L.* sp44 complex; however, $GMYC_{rbcL}$ split *L. sonderi* and into two PSHs. As in *cox*3, the $ABGD_{rbcL}$ result produced a greater number of PSH compared to GMYC by splitting *L.* sp44, *L. schneideri,* and *L. undulata* into two PSH each; however, $ABGD_{rbcL}$ merged *L.* sp78 and its closest relative *L.* sp. (EU579955) into one PSH. PTP_{rbcL} results merged the *L.* sp21 complex with *Lobophora gibbera*, and in agreement with the $GMYC_{rbcL}$ results, resolved the *L.* sp44 complex and *L. schneideri* as a single PSH. In all *rbc*L SDM *L. canariensis* and *L.* sp4 grouped as a single PSH (Fig. 1).

Analyses of *psb*A were more conserved than *cox*3 and *rbc*L across all three SDM, and identified 14 (GMYC/PTP) or 17 (ABGD) PSH. *L. guadeloupensis*, *L. littlerorum*, *L.* sp64, and *L.* sp86 were the species without *psb*A data. $GMYC_{psbA}$ and PTP_{psbA} recognized *L. schneideri*, *L. declerckii*, *L. undulata*, *L. sonderi*, *L. variegata*, *L.* sp44 complex, *L.* sp40, *L.* sp58, *L.* sp65, *L.* sp77, *L.* sp78, and *L.* sp. (*LAF07637*) as a single PSH; however, both methods merged the *L.* sp21 complex with *L. gibbera* sequences (Fig. 1). Results from $ABGD_{psbA}$ differed from the other two SDM by recognizing *L.* sp21, *L.* sp22, and *L.* sp. (LAF 06736) as distinct PSH. In all SDM for *psb*A *L. canariensis* and *L.* sp4 grouped as a single PSH (Fig. 1).

Phylogenetic relationships. ML and Bayesian analyses of *cox*3, *rbc*L, and *psb*A markers resulted in similar topologies; thus only ML trees are shown in Figures S2–S4 in the Supporting Information but they display bootstrap values (ML) and posterior probabilities (BI). All *Lobophora* phylogenies, including the concatenated results, revealed two main lineages, a small clade A and a large clade B (Fig. 1, Figs. S2–S4) whose most recent common ancestor has been dated to 85 mya (Vieira et al. 2017). Clade A contains four of the eight herein described new species, and is composed so far of species found in the Western and Eastern Atlantic (including the Mediterranean), Indian, and Western Pacific oceans (Fig. 1, Figs. S2–S4). Clade B contains the remaining four newly described species, three of which are restricted to the EP and one to the WA. Clade B is the largest one in the *Lobophora* phylogenies, characterized by species found in different places around the world (Fig. 1, Figs. S2–S4).

Taxonomic conclusions. We identified 14 distinct species of *Lobophora* for the WA, including five new species herein described. In the EP Ocean, we identified five distinct species, three of which are new (Fig. 1). These eight new WA and EP *Lobophora* species are described below. Additional morphological and anatomical information about each species is provided in Table S4 in the Supporting Information. GenBank accession numbers are included within each specimen's description (in the case of the holotypes) and at the end of the voucher information (for the isotypes or additional specimens sequenced), in the following order: *cox*3, *rbc*L, *psb*A; missing data are indicated by a "–." Publicly available BOLD accessions are cited when sequences are not available through GenBank. Asterisks indicate herbarium specimens from which DNA was extracted directly rather than from a fragment of the specimen preserved in silica gel-desiccant.

Lobophora adpressa O.Camacho & C.Fernández-García sp. nov. (Fig. 2, A and B)

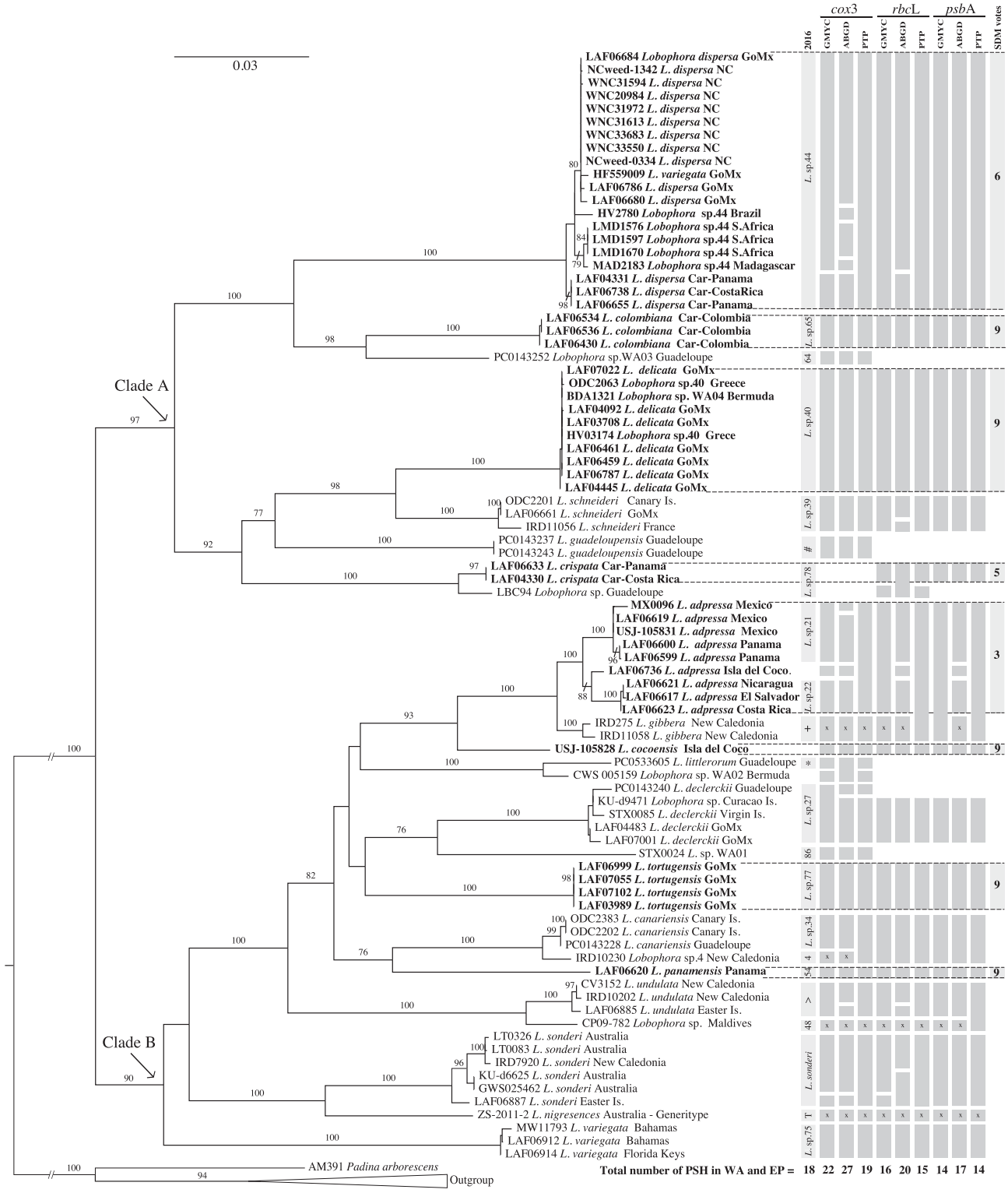

FIG. 1. Concatenated phylogeny (*cox*3, *rbc*L, and *psb*A) showing on left results of different species delimitation methods (GMYC, ABGD, PTP) corresponding to *Lobophora* species found in WA and EP (displayed in bold font), plus closer sister taxa. Total number of species found per gene/method shown at bottom. Outgroup taxa were *Dictyota dichotoma*, *Dictyopteris polypodioides,* and *Padina arborescens.* Abbreviations: **Car**, Caribbean; **GoMx**, Gulf of Mexico; **NC**, North Carolina USA; **S**., South; **SDM**, Species Delimitation Methods; **4**, *Lobophora* sp4; **48**, *L.* sp48; **58**, *L.* sp58; **64**, *L.* sp64; **86**, *L.* sp86; **#,** *L guadeloupensis*; +, *L. gibbera*, *, *L. littlerorum*; **>**, *L. undulata*; **T**, Generitype; **2006**, Vieira et al. 2016; **X**, Lineages or species not found yet in WA or EP.

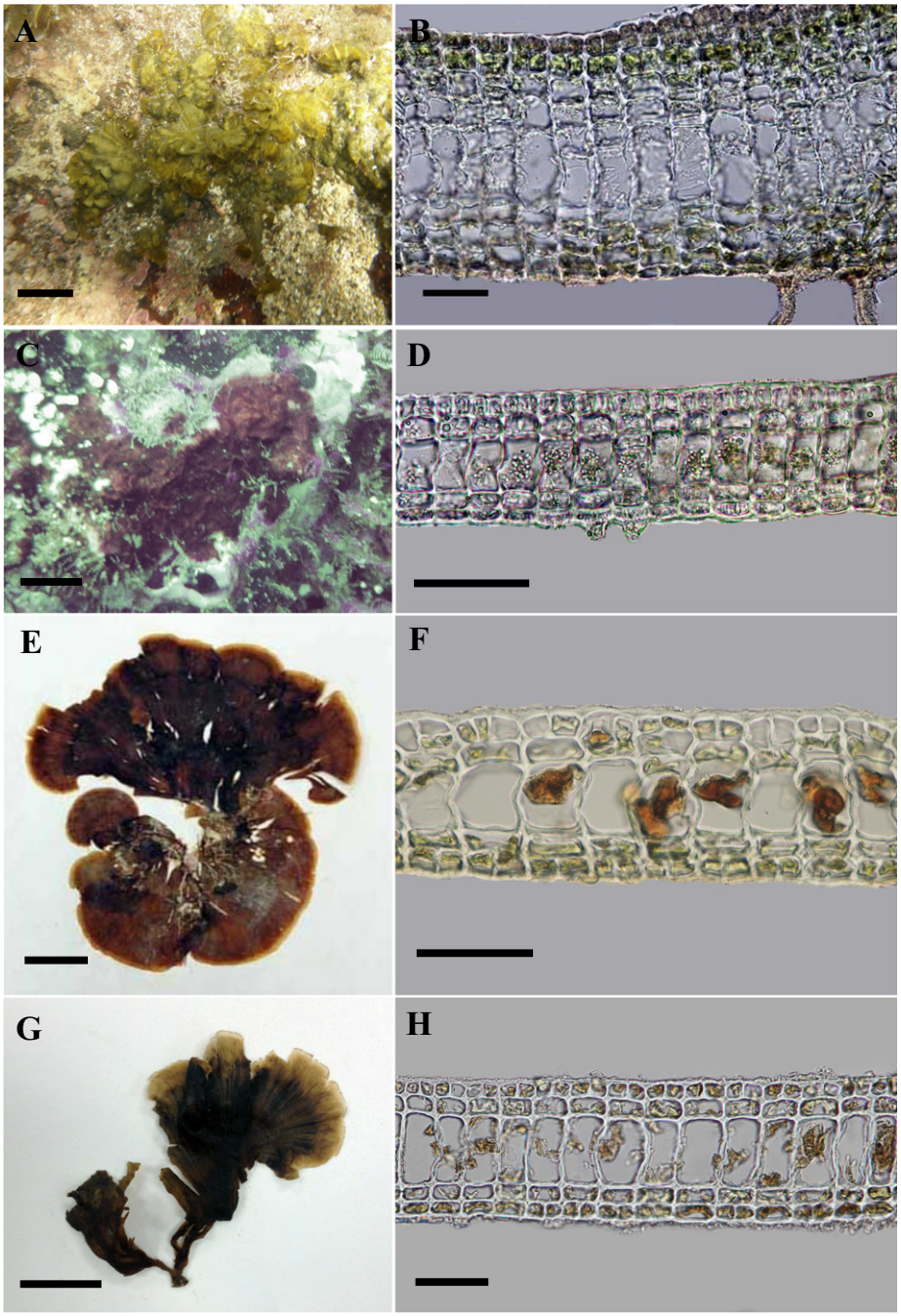

FIG. 2. Habit and corresponding transverse section through middle of thallus of A and B: *Lobophora adpressa*, C and D: *L. cocoensis*, E and F: *L. colombiana*, G and H: *L. crispata*, Scale bars A, C, E, G: 1 cm, B, D, F, H: 60 µm.

Description: Thallus dark brown with yellowish lines or spots, crustose, coarse with rugose surface (Fig. 2A), firmly attached to the substratum by abundant moniliform rhizoids on the entire ventral surface. Blades 139–277 µm thick, composed of 8–14 cell layers with a single to double cell-layered medulla surrounded by 3–6 and 3–8 layers of ventral and dorsal cortical cells respectively (Fig. 2B). Medullary cells 34–68 µm height and 24–51 µm width. Sporangia 32.2 µm in diameter, without paraphyses. GenBank accession numbers: KU364208$_{cox3}$, KU364169$_{rbcL}$, KU364254$_{psbA}$.

Holotype: USJ 105831, Mexico, Oaxaca, Puerto Angel, Playa El Panteón, growing on rocky-calcareous substratum, 1–3 m, 15.66403 N, 96.49542 W, coll. C. Fernández-García, 13.ii.2009.

Additional specimens examined: LAF 06599, Panama, Veraguas, Is. Cebaco, Sombrero Rock, 14 m, 7.48229 N, 81.25705 W, coll. B. Wysor & N. Hammerman, 13.v.2013, –, KU364166$_{rbcL}$, KU364251$_{psbA}$; LAF 06600, Panama, Veraguas, Is. Cebaco, Sombrero Rock, growing on rock, 14 m, 7.48229 N, 81.25705 W, coll. B. Wysor, 13.v.2013, KU364206$_{cox3}$, KU364167$_{rbcL}$, KU364252$_{psbA}$; LAF 06617, El Salvador, Los Cóbanos,

El Privado Beach, growing on rock, intertidal, 13.52722 N, 89.81133 W, coll. C. Fernández-García & A. Planas, 1.ii.2009, KU364207$_{cox3}$, KU364168$_{rbcL}$, KU364253$_{psbA}$; LAF 06736, Costa Rica, Isla del Coco, Islote Manuelita, growing on rock, 15–20 m, 5.56127 N, 87.04848 W, coll. C. Fernández-García, 18.vii.2013, MH885802$_{cox3}$, MH885828$_{rbcL}$, MH885817$_{psbA}$.

Additional sequenced specimens: LAF 06619 Mexico, Oaxaca Puerto, Escondido, growing on rock, 3–4 m, 15.8575 N, 97.06567 W, coll. C. Fernández-García, 17.ii.2009, KU364209$_{cox3}$, KU364170$_{rbcL}$, KU364255$_{psbA}$; LAF 06621 (Vieira et al. 2016).

Etymology: Named after the crustose or adpressed habit of the species, laying flat against the substratum.

Distribution: Tropical Eastern Pacific Ocean (Mexico; El Salvador; Nicaragua; Costa Rica: Isla del Coco; Panama).

Remarks: *Lobophora adpressa* includes *Lobophora* sp21 and *L.* sp22, recognized by Vieira et al. (2016, 2017) as two distinct species, plus LAF 06736. Results from different molecular-based approaches showed low levels of agreement among *L. adpressa* intra-specific lineages (Fig. 1, Figs. S2–S4). *Lobophora adpressa* intra-specific lineages presented overlapping morphologies (external habit and cell measurements), particularly between *L.* sp22 and LAF 06736. The number of cortical cell layers in specimens of this complex, a character useful to identify certain species, also showed some considerable variation (Table S4). Despite the observed intra-specific morphological variation, *L. adpressa* is the most morphologically distinct species compared to the other seven described in this study based on overall cellular dimensions and total number of cortical cell layers (Table S4). This species received nine votes (Fig. 1, Figs. S2–S4).

Lobophora cocoensis O.Camacho & C.Fernández-García sp. nov. (Fig. 2, C and D)

Description: Thallus dark brown, crustose (Fig. 2C), attached to the substratum by abundant rhizoids present on the entire ventral surface, except in apical (marginal) parts where it is loosely attached. Blades 62–87 μm thick, composed of 4–5 cell layers with a single cell-layered medulla surrounded by (one–) two ventral cortical cell layer(s) and two dorsal cortical cell layers (Fig. 2D). Medullary cells 21–36 μm height and 32–71 μm width. Sexual reproductive structures unknown. BOLD accession numbers: LOBP002-19cox3, LOBP002-19rbcL; GenBank accession number: MH885818$_{psbA}$.

Holotype: USJ 105828, Costa Rica, Isla del Coco, Islote Manuelita afuera, growing on rock, 15–20 m, 5.56127 N, 87.04848 W, coll. C. Fernández-García, 21.viii.2013.

Additional specimens examined: LAF 06737B (isotype), BOLD accession numbers LOBP003-19$_{cox3}$, –, LOBP002-19$_{psbA}$. LAF 06737C (isotype), –, –, –.

Etymology: This species is named for the collection area, Isla del Coco, Costa Rica.

Distribution: Eastern Pacific (Costa Rica: Isla del Coco).

Remarks: The newly sequenced specimens are from a single collection. Currently considered to be an endemic species for Isla del Coco, it received 15 votes (Fig. 1, Figs. S2–S4).

Lobophora colombiana O.Camacho & Fredericq sp. nov. (Fig. 2, E and F)

Description: Thallus brown, decumbent (Fig. 2E), attached to the substratum by rhizoids predominantly in basal portions. Blades 74–94 μm thick, composed of five cell layers with a single cell-layered medulla surrounded by two layers of cortical cells on both ventral and dorsal sides (Fig. 2F). Medullary cells 34–49 μm height and 20–28 μm width. Sexual reproductive structures unknown. GenBank accession numbers: KU364202$_{cox3}$, KU364162$_{rbcL}$, KU364247$_{psbA}$.

Holotype: LAF 06430, Colombia, Magdalena, Bahía Granate, growing on rock, intertidal to 3 m, 11.293877 N, 74.190816 W, coll. O. Camacho, 31.vii.2011.

Additional specimens examined: LAF 06536 (isotype), GenBank accession numbers: MH885804$_{cox3}$, MH885830$_{rbcL}$, MH885820$_{psbA}$.

Additional sequenced specimens: LAF 06534*, Caribbean Colombia, Magdalena, Granate bay, growing on rock, intertidal to 3 m, 11.293877 N, 74.190816 W, coll. O. Camacho, 31.vii.2011, MH885803$_{cox3}$, MH885829$_{rbcL}$, MH885819$_{psbA}$.

Etymology: This species is named for the collection area, Caribbean Colombia.

Distribution: Western Atlantic (Colombia).

Remarks: This species received 15 votes (Fig. 1, Figs. S2–S4) and corresponds to *Lobophora* sp65 of Vieira et al. (2016, 2017).

Lobophora crispata O.Camacho & Fredericq sp. nov. (Fig. 2, G and H)

Description: Thallus dark brown, fan-shaped, and decumbent (Fig. 2G), attached to the substratum by rhizoids, predominantly in basal parts, occasionally developing a stipe. Curled blades 100–122 μm thick, composed of (five–) six–seven cell layers with a single cell-layered medulla surrounded by two (–three), and two (–three) layers of ventral and dorsal cortical cells respectively (Fig. 2H). Medullary cells 32–63 μm height and 20–26 μm width. Sexual reproductive structures unknown. GenBank accession numbers: –, KU364149$_{rbcL}$, KU364230$_{psbA}$.

Holotype: LAF 04330A, Panama, Bocas del Toro, Big Plantain Cay, growing on rock, 15–20 m, 9.12376 N, 81.7943 W, coll. O. Camacho, 6.viii.2010.

Additional specimens examined: LAF 04330B (isotype), –, –, –. LAF 06633, Costa Rica, Limón, Parque Nacional Cahuita, growing on rock, 1 m, 9.73690 N, 82.80939 W, coll. C. Fernández-García, 29.ix.2012, –, MH885839$_{rbcL}$, MH885821$_{psbA}$.

Etymology: The species is named for its habit of crisped and curled blades.

Distribution: Western Atlantic (Panama; Costa Rica; Guadeloupe, F.W.I)

Remarks: This species received seven votes, this value being low as this species does not have *cox*3 data (Fig. 1, Figs. S2–S4). *Lobophora crispata* corresponds to *Lobophora* sp78 of Vieira et al. (2016, 2017).

Lobophora delicata O.Camacho & Fredericq sp. nov. (Fig. 3, A and B)

Description: Thallus green to brown, crustose, or decumbent (Fig. 3A), delicate, attached by rhizoids on ventral surface, with margins often loosely attached. Blades thin, 48–65 μm thick, composed of three–four cell layers with a single to double cell-layered medulla surrounded by one layer of cortical cells on both ventral and dorsal sides (Fig. 3B). Medullary cells 20–34 μm height and 25–29 μm width. Sporangia 40 μm in diameter, lacking paraphyses. GenBank accession numbers: KU364203$_{cox3}$, KU364163$_{rbcL}$, KU364248$_{psbA}$.

Holotype: LAF 06459, USA, NW Gulf of Mexico, Ewing Bank, 60–90 m, 28.08983 N, 91.026166 W, coll. S. Fredericq & O. Camacho, grown in vitro from rhodoliths collected on 26.viii.2011.

Additional specimens examined: LAF 06787, USA, NW Gulf of Mexico, Ewing Bank, growing on

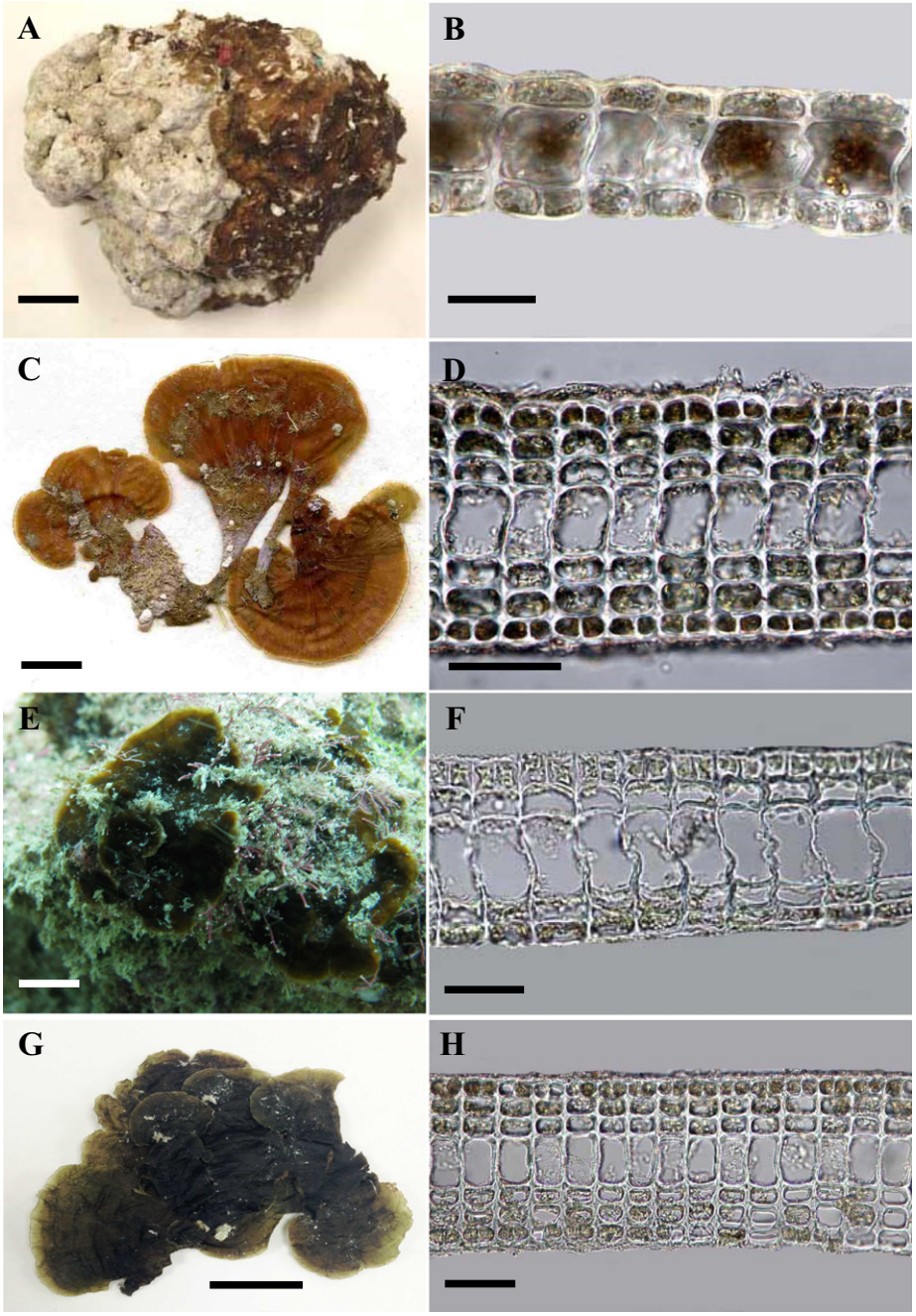

FIG. 3. Habit and corresponding transverse section through middle of thallus of A and B: *Lobophora delicata*, C and D: *L. dispersa*, E and F: *L. panamensis*. G and H: *L. tortugensis*. Scale bars A, C, E, G: 1 cm; B, F: 30 μm; D, H: 60 μm.

rhodoliths, 61 m, 27.965316 N, 91.241683 W, coll. S. Fredericq & O. Camacho, 19.x.2013, KU364219$_{cox3}$, KU364178$_{rbcL}$, KU364266$_{psbA}$; LAF 07022, USA, NW Gulf of Mexico, Ewing Bank, 90 m, 28.1000 N, 91.036833 W, coll. S. Fredericq & O. Camacho, grown in vitro from rhodoliths collected on 14.ix.2014, MH885805$_{cox3}$, –, –.

Additional sequenced specimens: LAF 04443, USA, NW Gulf of Mexico, Ewing Bank, growing on rhodoliths, 58–91 m, 28.10110 N, 91.03576 W, coll. S. Fredericq, 6.iv.2012, MH973851$_{cox3}$, MH973852$_{rbcL}$, –; LAF 03708; LAF 04092; LAF 04329; LAF 04445; LAF 06459; and LAF 06461 (Vieira et al. 2016).

Etymology: The epithet, *delicata* (L.), refers to the delicate nature of the thallus of the species, composed of only a few cell layers.

Distribution: Western Atlantic (Gulf of Mexico, Bermuda) and Mediterranean Sea (Greece).

Remarks: *Lobophora delicata* received 15 votes; it is the same as *L.* sp40 of Vieira et al. (2016, 2017). *Lobophora delicata*, *L. guadeloupensis* and *L. abaculusa* C.W. Vieira, Payri & De Clerck are the only species currently known to possess a single to double cell-layered medulla.

Lobophora dispersa O.Camacho, Freshwater & Fredericq sp. nov. (Fig. 3, C and D)

Description: Thallus light to dark brown, with bright and coarse appearance, fan-shaped, stipitate (Fig. 3C), attached to the substratrum by numerous rhizoids grown from the base. Blades 78–131 μm thick, composed of five–eight cell layers, with a single cell-layered medulla surrounded by two–three ventral cortical cell layers, and three–four dorsal cortical cells layers (Fig. 3D). Medullary cells 23–45 μm height and 18–28 μm width. Sexual reproductive structures not found. GenBank accession numbers: MH885813*cox3*, MH885837*rbcL*, MH885825*psbA*.

Holotype: WNC 33550, USA, North Carolina, Onslow Bay, growing on hard bottom, 23 m, 34.38835 N, 76.57057 W, coll. D.W. Freshwater & A. Alder, 29.x.2013.

Additional specimens examined: LAF 04331, Panama, Bocas del Toro, Tervi Bight, growing on rock, 9.425 N, 82.377 W, coll. S. Fredericq, 13.vii.2008, KU364189$_{cox3}$, KU364150$_{rbcL}$, KU364231$_{psbA}$; LAF 06655, Panama, Chiriqui, East of Rio Cañaveral, growing on rock, 1 m, 9.04494 N, 81.73882 W, coll. O. Camacho, 6.viii.2010, –, –, KU364259$_{psbA}$; LAF 06680*, USA, Off shore Louisiana, NW Gulf of Mexico, 63 m, 28.6169 N, 89.55533 W, coll. S. Fredericq, 28.vi.2016, KU364214$_{cox3}$, –, KU364262$_{psbA}$; LAF 06684*, USA, NW Gulf of Mexico, Ewing Bank, 59 m, 27.80798 N, 091.0357667 W, coll. S. Fredericq, 22.vii.2008, MH885806$_{cox3}$, –, –; LAF 06738, Costa Rica, Limon, Manzanillo, growing on dead coral, 4 m, 9.63632 N, 82.65609 W, coll. J. Nivia, 21.vii.2013, KU364216$_{cox3}$, KU364176$_{rbcL}$, KU364264$_{psbA}$; LAF 06786, USA, NW Gulf of Mexico, Ewing Bank, growing on rhodoliths, 90 m, 28.07723 N, 91.033566 W, coll. S. Fredericq & O. Camacho, 19.x.2013, KU364218$_{cox3}$, KU364177$_{rbcL}$, KU364265$_{psbA}$.

Additional sequenced specimens: WNC 20984, USA, North Carolina, Onslow Bay, growing on wreck, 20 m, 34.5451 N, 76.8950 W, coll. D.W. Freshwater & P. Whitfield, 12.ix.2012, MH885809$_{cox3}$, MH885833$_{rbcL}$, –; WNC 31594, USA, North Carolina, Onslow Bay, growing on hard bottom, 41 m, 33.5033 N, 77.1660 W, coll. D.W. Freshwater & B. Degan, 25.vi.2009, MH885810$_{cox3}$, MH885834$_{rbcL}$, –; WNC 31613, USA, North Carolina, Onslow Bay, growing on hard bottom, 27–28 m, 34.3929 N, 76.8987 W coll. D.W. Freshwater & B. Degan, 30.vi.2009, MH885811$_{cox3}$, MH885835$_{rbcL}$, MH885824$_{psbA}$; WNC 31972, USA, North Carolina, Onslow Bay, growing on hard bottom, 33-34 m, 33.8034 N, 77.3132 W, coll. J. Dorton, 26.x.2011, MH885812$_{cox3}$, MH885836$_{rbcL}$, –; WNC 33683, USA, North Carolina, Onslow Bay, growing on hard bottom, 33–34 m, 33.8034 N, 77.3132 W, coll. D.W. Freshwater & S. Hall, 15.viii.2012, MH885814$_{cox3}$, MH885838$_{rbcL}$, –; WNC 34156, USA, North Carolina, Onslow Bay, growing on hard bottom, 45 m, 33.6387 N, 76.9420 W, coll. D.W. Freshwater & R. Muñoz, 6.ix.2010, MH885808$_{cox3}$, MH885832$_{rbcL}$, MH885823$_{psbA}$; WNC 22852, USA, North Carolina, Onslow Bay, growing on hard bottom, 27 m, 34.1321 N, 77.3606 W, coll. D.W. Freshwater & G. Compeau, 06.ix.2013, MH885807$_{cox3}$, MH885831$_{rbcL}$, MH885822$_{psbA}$; LAF 06692*; LAF 06786; LAF 06997 (Vieira et al. 2016).

Etymology: The species epithet reflects the wide geographic distribution of this species.

Distribution: Western Atlantic (USA: North Carolina, Gulf of Mexico; Costa Rica, Panama, and Brazil) and Indian Ocean (South Africa; Madagascar).

Remarks: *Lobophora dispersa* received 11 votes and corresponds to *Lobophora* sp44 of Vieira et al. (2016, 2017). There were no significant morphological differences among specimens now considered part of this species.

Lobophora panamensis O.Camacho, C.Fernández-García, & Fredericq sp. nov. (Fig. 3, E and F)

Description: Thallus dark brown, crustose, firmly attached to the substratrum (Fig. 3E) by abundant rhizoids present along the entire ventral surface. Blades 100–121 μm thick, composed of five cell layers, a single cell-layered medulla surrounded by two layers of cortical cells on both ventral and dorsal sides (Fig. 3F). Medullary cells 43–59 μm height and 26–35 μm width. Sexual reproductive structures unknown. GenBank accession numbers: KU364210$_{cox3}$, KU364256$_{rbcL}$, KU364171$_{psbA}$.

Holotype: USJ 105830, Panama, Golfo de Panama, Archipiélago de las Perlas, Isla Balloneta, growing on rock, 1–3 m, 8.4875806 N, 79.0676472 W, coll. C. Fernández-García, 25.vi.2009.

Additional specimen examined: LAF 06620B (isotype), –, –, –.

Etymology: The species is named after the country where it was discovered, Panama.

Distribution: Eastern Pacific (Panama).

Remarks: *Lobophora panamensis* received 12 votes; it is the same as *Lobophora* sp58 of Vieira et al. (2016, 2017).

Lobophora tortugensis O.Camacho & Fredericq sp. nov. (Fig. 3, G and H)

Description: Thallus brown, crustose, or decumbent (Fig. 3G), attached to the substratrum by rhizoids predominantly in basal parts. Blades 94–102 μm thick, composed of (six–) seven cell layers with a single cell-layered medulla surrounded by two (–three) ventral cortical cell layers, and three dorsal cortical cell layers (Fig. 3H). Medullary cells 35–44 μm height and 22–25 μm width. Sexual reproductive structures unknown. GenBank accession numbers: KU364225$_{cox3}$, KU364185$_{rbcL}$, KU364272$_{psbA}$.

Holotype: LAF 06999, USA, SE Gulf of Mexico, Northwest of Dry Tortugas, growing on rhodoliths, 65 m, 24.81548 N, 83.67682 W, coll. S. Fredericq, 10.ix.2014.

Additional specimen examined: LAF 07102 (isotype), USA, SE Gulf of Mexico, Northwest of Dry Tortugas, 65 m, 24.81100 N, 83.67667 W, coll. S. Fredericq & O. Camacho, grown in vitro from rhodoliths collected on 10.ix.2014. MH885816$_{cox3}$, –, MH885827$_{psbA}$.

Additional sequenced specimens: LAF 03989, USA, offshore Louisiana, NW Gulf of Mexico, growing on rhodoliths, 63 m, 27.98265 N, 91.6520167 W, coll. S. Fredericq & O. Camacho, 5.xii.2010. –, –, KU364228$_{psbA}$. LAF 07055, USA, SE Gulf of Mexico, vicinity of Dry Tortugas, growing on rhodoliths, 60 m, 24.81548 N, 83.67682 W, coll. S. Fredericq & O. Camacho, grown in vitro from rhodoliths collected on 10.ix.2014. MH885815$_{cox3}$, –, MH885826$_{psbA}$.

Etymology: The species is named for its occurrence and here selected type locality, Dry Tortugas Natural Park, Florida.

Distribution: Western Atlantic (Gulf of Mexico).

Remarks: This species received 10 votes and corresponds to *Lobophora* sp77 of Vieira et al. (2016, 2017).

DISCUSSION

This study contributed to resolving *Lobophora* species in the Western Atlantic and the Eastern Pacific oceans, two regions that needed taxonomic attention, and baseline studies on this algal genus. Following a consensus approach using three molecular markers, different single-marker SDM, and morphological evidence, 14 distinct *Lobophora* species were identified, and eight species from these two oceans were newly proposed.

Molecular techniques are now producing an abundance of DNA sequence data to identify species that are much easier, faster, and increasingly less expensive to produce than formal taxonomic descriptions. As a result, many molecularly defined PSH remain unnamed especially in organisms with simple morphologies and plagued with homoplasies such as algae (e.g., *Lobophora*: Vieira et al. 2016, 2017; *Portieria*: Payo et al. 2013, *Ostreobium*: Sauvage et al. 2016). These molecularly identified but unnamed species have been dubbed the "dark taxa" by Page (2016). Six of the eight newly described species in this study, *L. colombiana*, *L. crispata*, *L. delicata*, *L. dispersa*, *L. panamensis*, and *L. tortugensis*, were already identified as PSH in a worldwide *Lobophora* diversity assessment based primarily on *cox*3 DNA sequences (Vieira et al. 2016, 2017). The seventh species, *L. adpressa*, once proposed as two distinct putative new taxa (*L.* sp21 and *L.* sp22 in Vieira et al. 2016), was herein described as a single species. The eighth species, *L. cocoensis*, a species so far known only from Isla del Coco, Pacific Costa Rica, was newly sequenced and described in this study.

Global species delimitation. The total number of PSH varied between methods and markers. This is probably not only a result of different markers or methods, but also of dissimilar taxa sampling. However, this is the first study to produce a global SDM analysis to include not only *cox*3 (see Vieira et al. 2016) but also *rbc*L and *psb*A data. The GMYC method yielded more conservative estimates than ABGD and PTP. The main discrepancies among the methods involved the following species: *Lobophora adpressa*, *L. dispersa*, *L. pachyventera*, *L. obscura* (= *L. crassa*), and *L. sonderi* (see Fig. 1, Vieira et al. 2016). These species represented collections spanning wide geographic distributions, and the wider the geographic scale of sampling, the lower the success of identification queries. This occurs because wide geographic sampling tends to increase intraspecific differences and to lower interspecific variation, shrinking in this way the barcode gap distances and compromising SDM (Bergesten et al. 2012).

C*ox*3 was the most variable marker compared to *rbc*L and *psb*A (Fig. S1), and hence provided a larger number of PSH (Table 1) by splitting more lineages (Fig. 1). This has been a consistent pattern across *Lobophora* studies (Sun et al. 2012, Vieira et al. 2014, this study). Similar results were also observed in another closely related genus, *Padina* (also Dictyotales) where the mean substitution rate for *cox*3 was nearly 5-fold faster than *rbc*L (Silberfeld et al. 2013). *Lobophora* species diversity could be underestimated using the *psb*A marker because of its low substitution rate (Fig. S1), a result also reported by Vieira et al. (2014) based on a smaller number of samples. In our SDM analyses, *psb*A resolved fewer species compared to *cox*3 and *rbc*L (Table 1, Fig. 1) by grouping *Lobophora* species previously recognized as distinct (e.g., *L. adpressa* and *L. gibbera*).

Species diversity in the Western Atlantic and Eastern Pacific oceans. Prior to molecular-based phylogenies, the broadly defined *Lobophora variegata* was the only known species of *Lobophora* recorded for both the WA and the EP (e.g., Taylor 1945, 1960, Dawson 1957, Earle 1969, Abbott and Hollenberg 1976, Norris and Bucher 1982, Schnetter and Bula-Meyer 1982, Schneider and Searles 1991, Littler and Littler 2000, Fredericq et al. 2009, Fernández-García et al. 2011, Wynne 2011, Norris et al. 2017). Recent molecular-based studies of Schultz et al. (2015) and Vieira et al. (2016) suggested that several species were passing under this name.

There are currently 14 molecularly distinct species of *Lobophora* known to occur in the tropical and temperate WA Ocean. Of the five PSH described by Schultz et al. (2015), four were validly described new species: *L. canariensis* (= *L. payriae*) *L. declerckii*, *L. guadeloupensis*, and *L. littlerorum*. The fifth species, "*L. variegata*" sensu Schultz et al. (2015), is being described as a new species, *L. schneideri*, elsewhere (Vieira et al. 2018). *Lobophora variegata* (type locality: Antilles, West Indies) was confirmed for the WA based on *cox*3, *rbc*L, and *psb*A sequences from type material (Vieira et al. 2016). Five of the remaining undescribed WA species of *Lobophora* are herein described as new, totaling 11 named *Lobophora* species in the WA. The three remaining lineages (*L.* sp64. *L.* sp86, and *L.* spWA02) of the 14 species known now to occur in the WA, were previously reported, but not formally described by Schultz et al. (2015), and remain undescribed. The lineage of *L.* sp (EU579955) from Guadeloupe (Bittner et al. 2008), and closely related to *L. crispata*, needs further molecular and morphological analysis to be recognized or not as a distinct species (Fig. 1).

Current molecular evidence has shown that *Lobophora variegata* does not occur in the EP (Vieira et al. 2016). Five distinct *Lobophora* species have been previously identified for the EP Ocean using molecular data: *L. sonderi*, *L. undulata,* and three undescribed PSH (Vieira et al. 2016, 2017). One of these undescribed PSH was here described as *L. panamensis*, and the remaining two PSHs were herein merged and described as a single new species, *L. adpressa*. While this merger reduced the number of molecularly recognized EP species to four, a previously unknown species was recently collected from Isla del Coco and here described as *L. cocoensis*, making the final number of EP *Lobophora* species still five.

Our conservative approach to species delimitation merged some distinct PSH (as recognized by different SDM) into single species. These taxa probably correspond to species-complexes that may be recognized as different taxa in the future. Examples of such *Lobophora* species complexes include *L. dispersa*, *L. adpressa,* and *L. sonderi* with at least two–three intraspecific clades (Fig. 1).

Phylogeny & biogeography. All five EP *Lobophora* species belong to the larger clade B, whereas WA species were present in both clades A and B (Figs. S2–S4; Schultz et al. 2015). Both the WA and EP *Lobophora* floras seem to have originated from a range of complex evolutionary scenarios that started taking place after the opening of the Atlantic Ocean and the closure of the Tethys Sea, ~85 million years ago (see Vieira et al. 2017 for a time-calibrated phylogeny). No evidence of substantial local radiations in either the WA or the EP was detected in any of our phylogenies (Fig. 1, Figs. S2–S4).

Most species were restricted to a particular biogeographic province or ecoregion, such as the Caribbean Sea (e.g., *Lobophora crispata*), the Gulf of Mexico (e.g., *L. tortugensis*), or both (e.g., *L. declerckii*). Species known so far from a single location (e.g., *L. cocoensis*, *L. colombiana*, *L. panamensis*) do not necessarily mean they represent endemic taxa as new records might be found elsewhere. On the other hand, *L. delicata* has a disjunct distribution with most collections coming from the Gulf of Mexico but it is also present in Bermuda and Greece. The extent of the distribution of this species remains unknown, and the Greece records could represent a case of anthropogenic introduction. Another case of widespread distribution includes *L. dispersa* whose range extends from North Carolina, USA, to Brazil, including South Africa and Madagascar (Fig. S5 in the Supporting Information). Although more detailed inferences about the biogeographic patterns and evolutionary relationships of WA and EP *Lobophora* species need further exploration, this study clarified the species and their known distributions.

Morphology. Despite overall habit similarities, our morphological analyses showed that vegetative characters were useful for differentiating most species. For example, thallus (blade) thickness was found to be diagnostic in all WA and EP species (Table S4), a character also diagnostic in some Indo-Pacific taxa (Vieira et al. 2014). Likewise, the number of cell layers in both the dorsal and ventral cortices, as well as in the medulla, were diagnostic in the WA species described by Schultz et al. (2015), and held true in this study. Other anatomical characters such as medullary cell height and length, and the length of subcortical cells were less valuable as diagnostic characters (Table S4). Reproductive structures are rare in *Lobophora* collections (Vieira et al. 2014, Schultz et al. 2015) and were only occasionally found in our specimens (e.g., *L. adpressa*, *L. delicata*). Over all, morphology helped distinguish *L. adpressa* (coarse crust, several cortical cell layers), *L. delicata* (delicate thallus, few cortical cell layers), and *L. dispersa* (coarse texture, fan-shaped, stipitate). Nevertheless, morphology alone does not always resolve species identifications and even in the most careful studies some species may remain cryptic or pseudo-cryptic (e.g., *L. colombiana* morphology can overlaps with *L. crispata*). For this reason, we suggest that to accurately identify *Lobophora* species

the best approach is to first generate DNA sequences (preferably *cox*3 and *rbc*L), followed by morphological and geographical analyses.

With the exception of *L.* sp64, *L.* sp86, and *L.* sp WA02, all the molecularly identified *Lobophora* PSH known for the WA and the EP have now been formally described. The three remaining undescribed species were originally recognized by Schultz et al. (2015) and were not found in our extensive field and herbarium collections. It is hoped that our contribution will lessen the harmful effects of "dark taxa" sensu Page (2016) with respect to marine macroalgae diversity. However, our knowledge of *Lobophora* species richness in the WA and EP oceans is a work in progress. Several marine locales and habitats along the Atlantic and Pacific America remain unexplored such as Brazil, Belize, the Guianas. Thus, future work based on the same markers herein implemented should reveal new, overlooked and/or cryptic *Lobophora* taxa in the WA, EP, and elsewhere. Investigations of new *Lobophora* species should be accompanied by formal taxonomic descriptions so that the gap between molecularly recognized taxa and officially named species decreases, including the current ~80 worldwide unnamed species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Figure S1. Box plot depicting the pairwise divergence by gene length of *psb*A, *rbc*L, and *cox*3 in *Lobophora* taxa downloaded from GenBank and from new datasets.

Figure S2. Maximum Likelihood *cox*3 phylogeny of all *Lobophora* species sequenced to date displaying both bootstrap values (BS) and posterior probabilities (PP). BS equal or above 60 and PP equal or above 0.6 are shown. Well-supported clades are considered those with values equal or above 70 BS and 0.7 PP. Newly described species in this study are shown in red; other species/lineages present in the Western Atlantic and Easter Pacific oceans appear in blue. *Lobophora crispata* is absent in the *cox*3 dataset and phylogeny.

Figure S3. Maximum Likelihood *rbc*L phylogeny of all *Lobophora* species sequenced to date displaying both bootstrap values (BS) and posterior probabilities (PP). BS equal or above 60 and PP equal or above 0.6 are shown. Well-supported clades are considered those with values equal or above 70 BS and 0.7 PP. Newly described species in this study are shown in red; other species/lineages present in the Western Atlantic and Easter Pacific oceans appear in blue.

Figure S4. Maximum Likelihood *psb*A phylogeny of all *Lobophora* species sequenced to date displaying both bootstrap values (BS) and posterior probabilities (PP). BS equal or above 60 and PP equal or above 0.6 are shown. Well-supported clades are considered those with values equal or above 70 BS and 0.7 PP. Newly described species in this study are shown in red; other species/lineages present in the Western Atlantic and Eastern Pacific oceans appear in blue.

Figure S5. Map showing the geographic distribution of the new eight Western Atlantic and Eastern Pacific species described in this study.

Table S1. List of specimens included in the concatenated analysis with their corresponding voucher number, reference, and GenBank accession numbers for *cox*3, *rbc*L, and *psb*A. Sequences in bold were newly produced in this study. Sequences marked with an asterisk (*) are less than 200 bp, and were deposited in BOLD database.

Table S2. Primers, PCR, and sequencing conditions used in this study. Temperature (T) is shown in Celsius degrees, and time in minutes. Primers were published by Draisma et al. (2001), Yoon et al. (2002), Kogame et al. (2005), Bittner et al. (2008), Mattio et al. (2008), Silberfeld et al. (2013), and Vieira et al. (2016).

Table S3. Number and percentage of *cox*3, *rbc*L, and *psb*A *Lobophora* sequences included in all phylogenetic and delimitation analyses after the removal of identical haplotypes.

Table S4. Average, minimum, and maximum values of morphological/anatomical measurements from the eight newly recognized *Lobophora* species from the Western Atlantic and Eastern Pacific. Measurements are expressed in micrometers (μm). Abbreviations: Min, minimal values; Max, maximal values; n, number of measurements/cross sections each from a different individual; n_T, number of transversal cross sections; $n_{L,}$ number of longitudinal cross sections; *, Height ventral/dorsal subcortical cells in *L. delicata* correspond to the height of cortical (more external) cells, subcorticals not present.